Determination of the optimal conditions for bovine serum albumin surface enhanced Raman scattering on silver colloids and nanoparticle films

Joell D. Reyes and Brian D. Gilbert
Department of Chemistry, Linfield College, McMinnville, Oregon 97128

Introduction
In proteomic studies, surface enhanced Raman scattering (SERS) has been used for protein identification. SERS is a sensitive surface technique used to identify various molecules through the enhancement of inelastic scattering. Raman scattering occurs when a molecule absorbs a photon and then returns to its original state, emitting a photon at a different frequency. Raman scattering is sensitive to the vibrational modes of the molecule, allowing for the identification of specific functional groups. The enhancement of inelastic scattering is critical for SERS because it increases the signal-to-noise ratio, allowing for the detection of molecules at lower concentrations. Raman scattering is used extensively in studies of biomolecules, including proteins, because of its high sensitivity and specificity.

Raman scattering occurs when a molecule absorbs a photon and then returns to its original state, emitting a photon at a different frequency. The vibrational modes of a molecule, including the stretching and bending vibrations of functional groups, are probed in the Raman spectrum. The intensity and frequency of these vibrations are characteristic of the molecule, allowing for its identification and quantification.

Materials and methods
Silver Colloid Solution
BSA, sodium citrate, and AgNO₃ were used as purchased from Sigma Aldrich (St. Louis, Missouri). Silver colloids were made with AgNO₃, sodium citrate (3%), and H₂O (1%) following Lee & MacIntyre. BSA (0.05 g) was dissolved in a phosphate buffer (100 mL). BSA solution (0.1 mL) was pipetted into a micro well plate followed by Na₂SO₄ (0.5 M, 0.1 mL) and silver colloids (0.1 mL). The mixture was exposed for 1-3 h at a wavelength of 530 nm. 600 g/mm, 50 µm slits, and a detector slit width of 0.80 were used.

Nanoparticle Films
Silver nanoparticle film preparation was adapted from NA. Small glass pieces were soaked in piranha (4 parts 35 % H₂O₂ and 1 part conc H₂SO₄) for 48 hours. Slides were rinsed with deionized water and soaked in colloid solution for 24 hours. The pH of the deionized water and colloid solution (4 part methanol: 1 part 35 % H₂SO₄) for 48 hours. Slides were rinsed with deionized water and soaked in colloid solution for 24 hours. The pH of the deionized water and colloid solution (4 part methanol: 1 part 35 % H₂SO₄) for 48 hours. Slides were rinsed with deionized water and soaked in colloid solution for 24 hours.

Instrumentation
Homebuilt Raman microscope was used to obtain SERS spectra. Laser - Spectra Physics, Excelsior, 532 nm, 150 mW. Microscope - Leica DMLM. 20X objective, 2000X Objective - Olympus Optical 540 DRLP. Slides for Raman Imaging - 25 mm Grade, 25 mm. Monochromator - CVI Digikrom 240, 600 g/mm grating, 50 mm s/d slits. Detection - Apogee thermoelectrically cooled CCD.

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Results
Figure 2. Raman spectrum of pure BSA with 10 second exposure.
Table 1. Tentative peak assignments from Figure 2.

<table>
<thead>
<tr>
<th>Raman Shift (cm⁻¹)</th>
<th>Assignments</th>
</tr>
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<tbody>
<tr>
<td>752</td>
<td>tyrosine (Tyr)</td>
</tr>
<tr>
<td>854</td>
<td>tyrosine (Tyr)</td>
</tr>
<tr>
<td>1165</td>
<td>peptide (Phe)</td>
</tr>
<tr>
<td>1297</td>
<td>alpha-helix 3</td>
</tr>
<tr>
<td>1450</td>
<td>tyrosine or phthaldialdehyde</td>
</tr>
<tr>
<td>1545</td>
<td>alpha-helix 3</td>
</tr>
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</table>

Figure 3. SERS spectra of BSA solution with varying pHs (Its descending order, 10, 6, 4, and 2).

Figure 4. SERS spectra of BSA (pH 4) with 2 second exposure on colloid solution.
Table 2. Tentative Raman shift assignments for the bands shown in Figure 4.

<table>
<thead>
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<th>Raman Shift (cm⁻¹)</th>
<th>Assignments</th>
</tr>
</thead>
<tbody>
<tr>
<td>835</td>
<td>tyrosine (Tyr)</td>
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<tr>
<td>1060</td>
<td>tyrosine (Tyr)</td>
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<tr>
<td>1165</td>
<td>peptide (Phe)</td>
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<tr>
<td>1297</td>
<td>alpha-helix 3</td>
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<tr>
<td>1367</td>
<td>tyrosine (Tyr)</td>
</tr>
<tr>
<td>1574</td>
<td>tyrosine (Tyr)</td>
</tr>
</tbody>
</table>

Figure 5. SERS spectrum of BSA on silver nanoparticle film.

Conclusions
Surface enhanced Raman scattering could be used to investigate label free detection of proteins. This is important for the identification of new proteins and a better understanding of protein interactions in their existing environments.

Acknowledgments
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For further information
Please contact bgilber@linfield.edu or bgilber@linfield.edu. More information on this can be found in Linfield College, McMinnville, Oregon 97128.

Literature cited